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ULTRASTRUCTURAL LOCALIZATION OF THIAMINE PYROPHOSPHATASE ACTIVITY IN EPITHELIOCYTES IN DUODENAL BIOPSY MATERIAL IN PEPTIC ULCER

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The presence of a developed thiamine pyrophosphatase (TPPase) system in the epithelial cells of the gastrointestinal tract regulates the supply of thiamine (vitamin B_1) to the tissues [4]. An important role in the metabolism and conversion of thiamine is played by the small intestine, in the mucosa of which this vitamin is absorbed [11, 12, 14]. However, the ultrastructural localization of TPPase activity has been studied in the epithelial cells of the small intestine mainly in experimental animals [13, 15, 16]. In peptic ulcer, as in other diseases of the gastrointestinal tract, thiamine absorption is disturbed [1, 2]. The chief results of investigations of thiamine metabolism in peptic ulcer have been obtained by biochemical methods [3, 8]. The use of electron-microscopic cytochemistry to investigate lysosomal enzymes, and TPPase in particular, in human cells in pathology [7] can shed light on the pathogenesis of several diseases at the subcellular level. There have been few such investigations in peptic ulcer [9].

The aim of this investigation was the ultrastructural cytochemical determination of the localization of TPPase activity in the epitheliocytes of the human small intestine in patients with peptic ulcer in phases of exacerbation and remission.

EXPERIMENTAL METHOD

An electron-cytochemical study was made of the TPPase content in biopsy material from the duodenal mucosa obtained from patients with peptic ulcer (men aged 25-45 years). Gastrointestinal fiberendoscopes (Olympus Optical Co., model JF type B_2 and B_3, side-viewing endoscopes, and type K forward viewing endoscope) were used for the endoscopic investigation of the mucosa of the gastrointestinal tract. According to the results of the endoscopic and histopathological investigations 17 patients had ulcers of the duodenal bulb in the remission phase, 13 had ulcers of the bulb in the exacerbation phase, and nine had superficial gastritis without any changes in the mucosa of the small intestine. Biopsy material was obtained from the mucosa of the visually least altered areas of the descending part of the duodenum for ultrastructural cytochemical and electron-microscopic investigation. Biopsy material was taken at endoscopy by the use of forceps contained in the duodenoscope outfit. Tissue sam-

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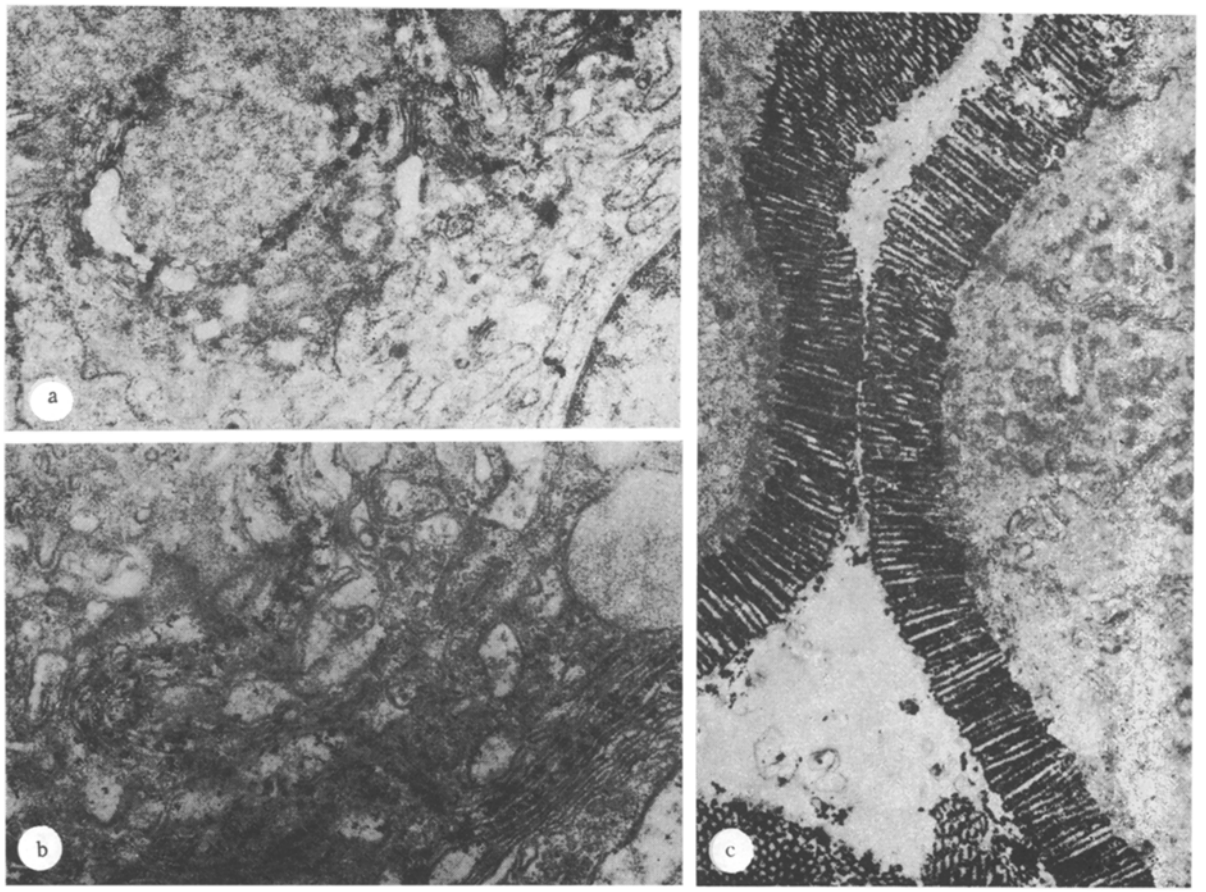

Fig. 1. Electron-cytochemical determination of TPPase in epitheliocytes of biopsy material from unchanged duodenal mucosa. a) Moderate TPPase activity on membranes of GLC (20,000 ×); b) increase in TPPase activity on membranes of GLC 1 h after administration of 5 mg thiamine (8000 ×); c) high TPPase activity in microvilli of brush border 1 h after administration of 60 mg thiamine (6000 ×). Here and in Figs. 2 and 3, the lead method of Novikoff and Goldfischer was used.

ples were obtained before administration of thiamine, and again 1 h after administration of 5 mg of thiamine per os (the physiological dose of the vitamin) or 60 mg intramuscularly (the therapeutic dose). Some of the biopsy material was fixed in a 4% solution of paraformaldehyde, made up in phosphate buffer, pH 7.2. After dehydration, the samples were embedded in a mixture of Epon and Araldite. Ultrathin sections were cut on the LKB Ultratome and stained with uranyl acetate and lead citrate by Reynolds' method. Another part of the biopsy material was used for ultrastructural cytochemical determination of TPPase by the lead method of Novikoff and Goldfischer [5]. Activity of the enzyme in ultrathin sections was assessed from the intensity of deposits of granules of the reaction product. The investigation was conducted in the JEM 100B electron microscope.

EXPERIMENTAL RESULTS

The general structural plan of the epithelial cells was preserved in biopsy material from the unchanged duodenal mucosa. The brush border consisted of tightly packed microvilli. Most of the epithelial lining consisted of cylindrical cells with a well developed cytoplasm, convoluted lateral plasma membranes, and numerous mitochondria. In some enterocytes a cytoplasmic reticulum and sacs of the Golgi lamellar complex (GLC) could be clearly distinguished. In the Goblet cells most of the cytoplasm was occupied by globules of mucus, and elements of a developed GLC also were found.

A moderate number of granules of reaction product was discovered in biopsy material incubated in medium with TPP only on the membranes of GLC (Fig. 1a).

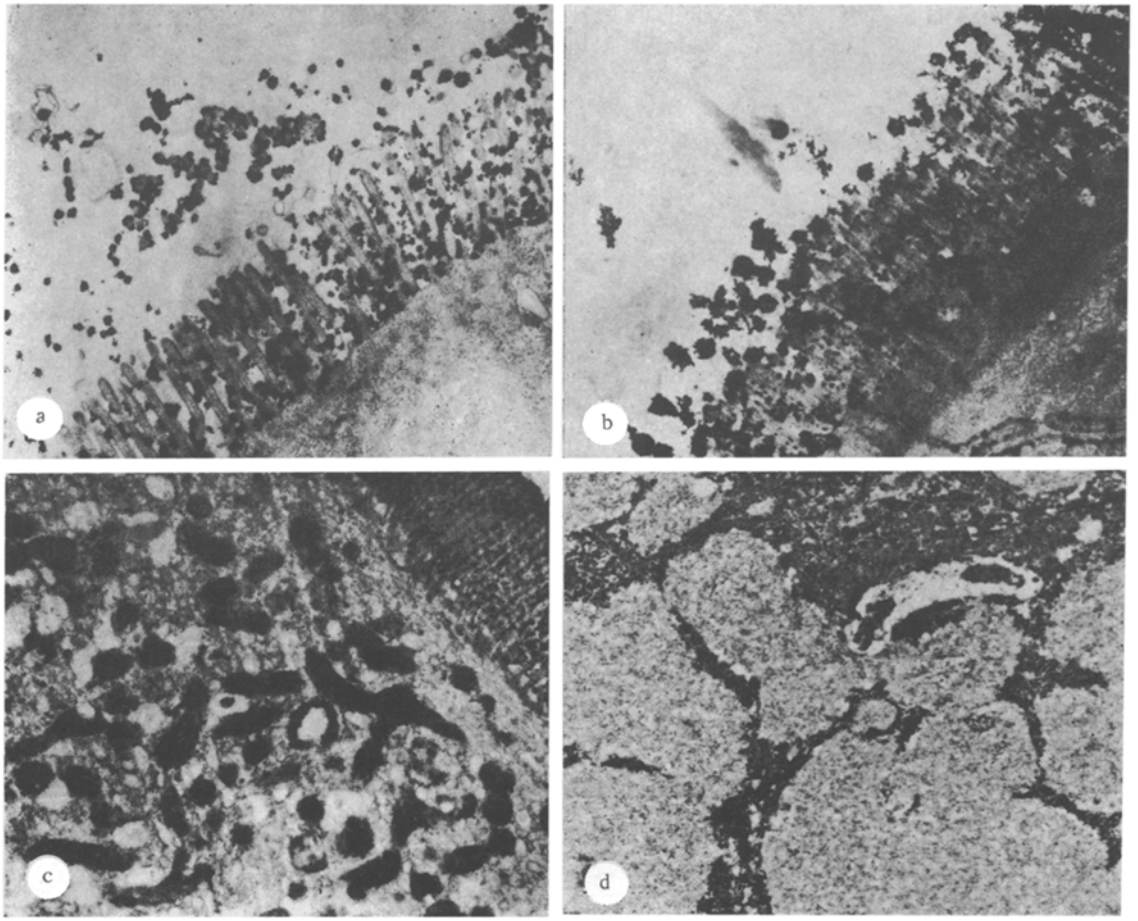

Fig. 2. Electron-cytochemical determination of TPPase in epitheliocytes of duodenal biopsy material during exacerbation of peptic ulcer. a) High TPPase activity on microvilli of brush border (8000 ×); b) greatly enhanced TPPase activity on microvilli of brush border and in zone of lateral plasmalemma (5000 ×); c) absence of intracellular TPPase activity and high activity on microvilli of brush border 1 h after administration of 5 mg thiamine (10,000 ×); d) increased TPPase activity on membranes of GLC and cytoplasmic reticulum in a Goblet cell 1 h after administration of 60 mg thiamine (10,000 ×).

After administration of 5 mg of thiamine per os the intracellular TPPase activity was increased: an abundance of granular material was found mainly on the membranes of GLC (Fig. 1b). After intramuscular injection of 60 mg thiamine, a positive reaction was found in biopsy material from this group, both inside and outside the cell (Fig. 1c). Many granules of reaction product were located on villi of the brush border of the epitheliocytes, and single, small granules were located inside the cell on the membranes of GLC and of the cytoplasmic reticulum.

Hyperemia, edema, and moderate polymorphic inflammatory-cellular infiltration of the mucosa were found in the biopsy material taken from patients with peptic ulcer in the exacerbation phase. These changes were most marked in the stroma of the villi.

The epithelium of the villi frequently showed signs of degeneration, the brush border lost the regular arrangement of its microvilli in some places, and foci of their complete destruction were found. In these cases products of the reaction for TPPase were distributed haphazardly on the microvilli of the brush border and in the zone of the lateral plasmalemma (Fig. 2a, b).

After administration of 5 mg of thiamine there was no intracellular reaction, but an even greater number of granules was found on the microvilli of the brush border (Fig. 2c). Administration of 60 mg of thiamine induced TPPase activity both extra- and intracellularly. The reaction products were found, in the form of an abundant granular deposit, on the microvilli of the brush border, and granules of reaction product also appeared in single entero-

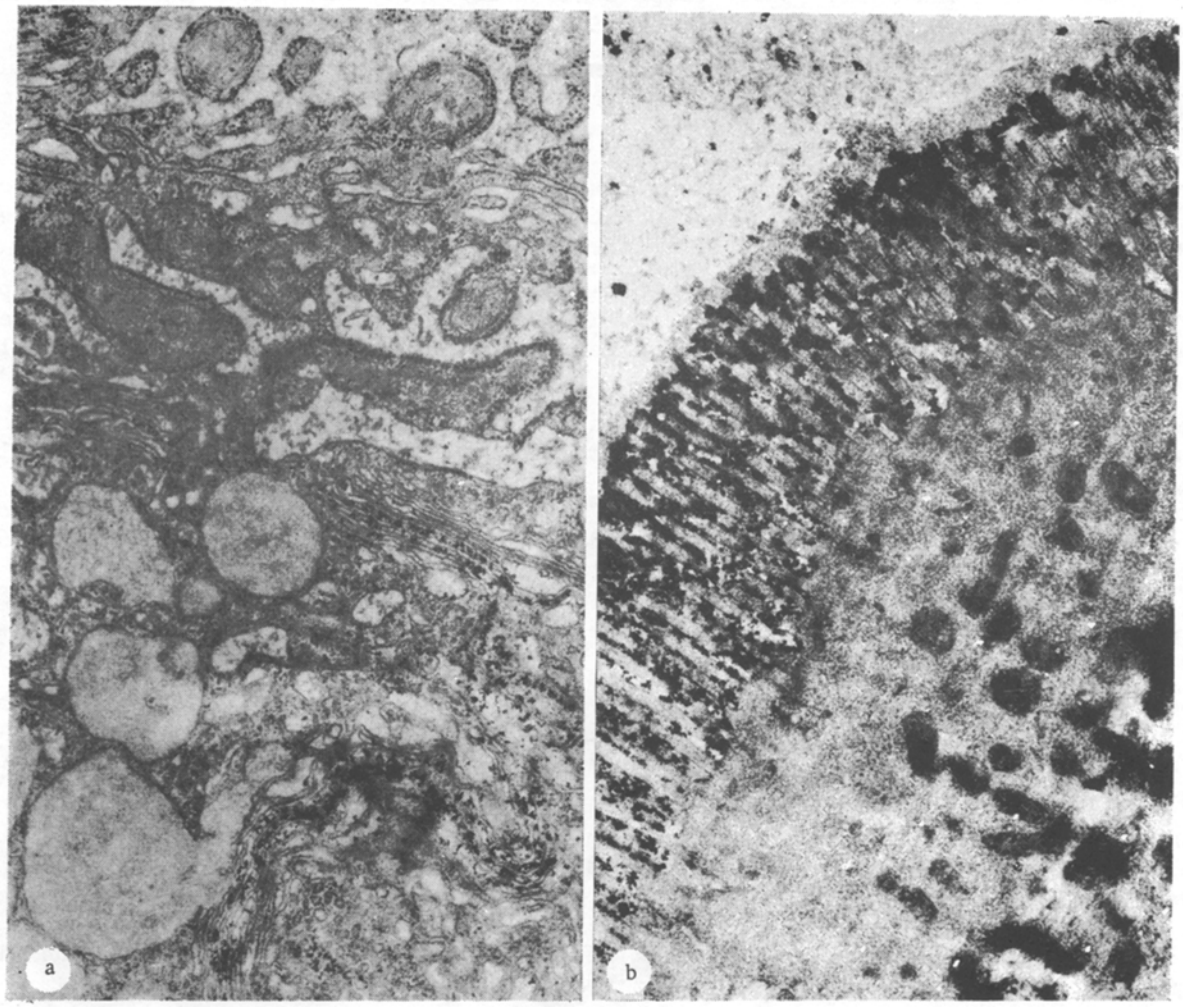

Fig. 3. Electron-cytochemical determination of TPPase in duodenal epitheliocytes from peptic ulcer patient in the remission phase. a) High TPPase activity on membranes of GLC 1 h after administration of 5 mg thiamine; b) sharply increased TPPase activity in brush border 1 h after administration of 60 mg thiamine. 10,000 ×.

cytes. The appearance of the enzyme on membranes of GLC and the cytoplasmic reticulum of the Goblet cells should be noted (Fig. 2d), evidence of the possible excretion of thiamine in the form of a complex with the secretory component of the glandulocytes.

In biopsy material from peptic ulcer patients in the remission phase the mucosa of the descending part of the duodenum preserved its general structure but the shape of the villi varied, and they were covered with cylindrical and goblet cells. The structure of the brush border was regular. In the ultrastructural organization of the enterocytes hyperplasia of the elements of GLC was observed, and the latter in some cells occupied the greater part of the perinuclear zone. Rouleaux and vesicles of this organelle were seen close to the lateral plasmalemma and in the apical and basal parts of the cell. The number of mitochondria was increased in the cylindrical cells, and the mitochondria were concentrated most frequently in the apical zone of the cytoplasm. The reaction for TPPase revealed numerous round granules of the enzyme on the membranes of GLC.

After administration of 5 mg thiamine to patients with peptic ulcer in the remission phase the intracellular TPPase activity rose considerably: an abundant granular deposit appeared in the region of the structures of GLC (Fig. 3a). Only solitary granules of the reaction product were found on the brush border. Administration of 60 mg thiamine was followed by a positive reaction for TPPase both on the brush border (Fig. 3b) and intracellularly — in the form of an abundant granular deposit on the membranes of GLC.

The localization and activity of TPPase thus differed depending on the state of the duodenal mucosa and also on the quantity of thiamine given and the method used.

In the unchanged or relatively unchanged mucosa (peptic ulcer in the remission phase) a positive intracellular reaction for TPPase was observed in the enterocytes, and its activity increased after administration of 5 mg thiamine, evidence of the enhanced metabolism and transcellular transport of the vitamin in this situation [4]. With an increase in the dose of the vitamin there was a tendency for activity of the enzyme in the duodenal cells to decrease. This may be explained on the grounds that after intramuscular injection of the vitamin it was largely transformed in the cells of other organs and tissues [6, 10].

In the presence of marked changes in the mucosa (peptic ulcer in the exacerbation phase) the enzyme could not be found intracellularly in the epitheliocytes, a situation regularly associated with destructive inflammatory changes in the integumentary cells of the small intestine.

The results of this investigation are evidence that, in the unchanged duodenal mucosa before and after administration of 5 mg thiamine, active transport of thiamine takes place from the lumen of the small intestine into the enterocytes, where it undergoes further metabolic transformation with the participation of TTPase (signs of accumulation of phosphoric esters of thiamine on the brush border appeared only after intramuscular injection of 60 mg thiamine). In the remission phase of peptic ulcer TPPase appeared on the brush border of the duodenal epithelial cells after administration of 5 mg thiamine. During exacerbation of peptic ulcer, the TPPase on the brush border preserved its activity in all cases, and this could be a compensatory and adaptive mechanism [9].

The extracellular activity of TPPase is evidently connected with disturbance of absorption of thiamine and its derivatives as a result of damage to the epithelial lining of the mucosa. This process is probably intensified also as a result of excretion of the excess of thiamine after intramuscular injection of a large dose of the vitamin.

Comparison of the data on the intra- and extracellular localization of TPPase in the duodenal mucosa and the levels of activity of the enzyme in different phases of peptic ulcer indicates that the character of metabolism of thiamine in the remission phase of the pathological process does not differ significantly from that in the unchanged duodenal wall. In the exacerbation phase of peptic ulcer thiamine absorption through the epithelial barrier is considerably disturbed and its intracellular transformation is modified.

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